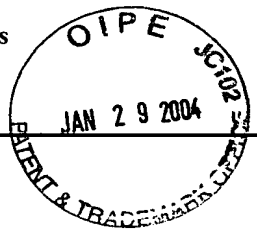
CERTIFICATE OF MAILING BY "EXPRESS MAIL" (37 CFR 1.10) Applicant(s): **Zimmerman, et al.**			Docket No. **113737.6**
Serial No. **09/762,850**	Filing Date **13 April 2001 (371 Date)**	Examiner **David M. Naff**	Group Art Unit **1651**

Invention: **Method For Producing Ultra-Pure Alginates**



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Declaration under 37 CFR 1.132
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09/762,850

Attorney's Docket No. 113737.6 ***Patent***

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Zimmermann, et. al.	Group No.:	1651
Serial No.:	09/762,850	Examiner:	David M. Naff
Filed:	April 13, 2001		
For:	Method for Producing Ultra-Pure Alginates		

SUPPLEMENTAL REPLY UNDER 37 C.F.R. § 1.111

DECLARATION UNDER 37 C.F.R. § 1.132

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Sir:

In response to the Advisory Action mailed on September 2, 2003, which followed Applicant's response to the Office Action mailed on May 6, 2003, in the subject application, Applicants respectfully submit the following Declaration.

Applicants respectfully request that this Declaration be appended to the Request for Continued Examination, which was submitted on November 6, 2003 in the subject application.

AUTHORIZATION

Applicants believe that no fees or extension of time are required for this submission. However, in the event that an extension of time is required, Applicants hereby submit a petition for such extension of time as may be necessary to make this response timely. The Commissioner is hereby authorized to charge any necessary fees to deposit account No. 502194. A duplicate of this Authorization is enclosed.

Respectfully Submitted,

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Attorney's Docket No. 49865.2 *Patent*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Zimmermann et. al

Serial No.: 09/762850

Filed: April 13, 2001

For: Method for Producing Ultra-pure Alginates

Group No. : 1651

Examiner: David M. Naff

DECLARATION OF Dr. Frank Thürmer
UNDER 37 C.F.R. § 1.132

I, Dr. Frank Thürmer, am a researcher. I received my PhD in natural science from the Bayerischen Julius-Maximilians-Universität Würzburg in 1998.

I am the head of production at CellMed AG and head the encapsulation and biopolymer team. I have extensive experience in the field of biotechnological, biomedical and biophysical research. Prior to the current employment I was 3 years the responsible project leader of the team "immobilization artificial organ replacement" at the Department of Biotechnology at the University of Würzburg. I headed several major grant projects and I am the author of about 15 publications, concerning among other thinks the properties of alginate.

I am familiar with the process and product that is disclosed and claimed in the above-captioned U.S. Patent application, and I consider myself skilled in the art pertaining to this application. I declare the following in support of the Request for Continued Examination submitted on November 6, 2003, in the above-captioned application.

1. The Nature of Alginate Salts

Alginates are soluble as salts only if the corresponding cation is monovalent (e.g. Na- or K- alginate). Alginates are not soluble as acids or as salts of multivalent cations (e.g. Ba- or Ca-alginates). The reason for this lack of solubility is the fact that with multivalent cations the alginate polymer chains are connected with ionic bonds. Further, alginates have a higher affinity with multivalent cations than with monovalent cations.

Multivalent cation salts of alginates can be dissolved by adding a complex-forming agent such as EDTA to remove the multivalent cations, while supplying surplus monovalent cations to form a new soluble salt with the alginate. An adequate concentrated EDTA solution for example will dissolve Ca- and Ba-alginate, whereas for example a citric acid-solution will only dissolve Ca-alginate.

2. Differences between the present invention and the prior art

A. Precipitation

According to DE 42 04 012 (and Klöck et al. 1994), the starting material is dissolved Na alginate (see DE 42 04 012, column 3, line 47, column 4, line 18, Klöck et al., page 640, left column, § 3, 1st sentence). Addition of BaCl in these prior processes yielded Ba-alginate particles, which are insoluble (see above) and therefore can be separated from the solution. The aim of this prior art separation was an attempt to separate the alginate from mitogenic substances. Because the earlier results with the prior technique still contain disadvantages (see examples in the present application), the present invention has been developed.

In contrast to the prior techniques, the invention described in the present application teaches in a first step dissolving the alginate from the plant material, in particular from the cellwalls. In the plants (in the raw material), the alginates are bound with multivalent cations.

Therefore, a complex forming agent is used, which has a higher affinity to the multivalent cations than does the alginate. An example of such an agent is EDTA. As the result of this step an alginate can be dissolved by removing the multivalent cations. The alginate is thus contained in the solution in a dissolved condition, and it can be separated from the solid plant components by filtration in the next step, the alginate being dissolved in the filtrate.

To make it quite clear, it is not the alginate which is complexed by the complex forming agent. In solution EDTA is anionic, as is the alginate, and for this reason an EDTA/alginate complex cannot be formed. The complex forming agent complexes only differently charged compounds, i.e. cations. At the same time, monovalent cations become bound to the alginate, which thus becomes soluble. The monovalent cations are either contained in the plant material (cell walls) in a sufficient amount (note: The algae grow in salt water) or may be added, e.g. as in example 1of the specification in the form of sodium carbonate, or, as in example 2 of the specification, in the form of sodium EDTA (Ethylenenediamine tetraacetic acid tetrasodium salt) as complex forming agent. The suspension mentioned in example 1 results from using dry raw algae material for extraction, i.e. complete plant material with all soluble and insoluble components. During the extraction process the alginate goes into solution (because multivalent cations are bound to the complex forming agent and are exchanged for monovalent cations binding to the alginate) whereas the insoluble plant material such as cell walls etc. form a suspension in the alginate solution.

In commercial alginate the multivalent cations already have been exchanged for monovalent cations by extraction, and this is the reason why it is soluble in the absence of EDTA.

In DE 42 04 012, Barium is used for precipitating alginate, while in the present invention the complex forming agent is used for dissolving the alginate into the solution by removing multivalent cations. According to the present invention, the complex forming

agent does not form a complex with the alginates, which is clear from the further steps of the process.

B. Purification

The present invention is new over the references (DE 42 04 012 and Klöck et al.) concerning purification of the alginate. According to the prior techniques, the material precipitated with Ba^{++} is purified by subsequent steps. On the other hand, purification with the present invention is obtained just from filtration and precipitating with alcohol. According to the present invention, the alginate is purified by precipitating it out of the solution that was previously formed by adding the complex-forming agent. This precipitation is done by adding e.g. ethanol to the solution. The addition of ethanol to the aqueous solution results in the fact that the dissolved alginate cannot be kept in the dissolved condition any longer. The alginate is thus precipitated as a salt. In this sense, this precipitation step in the present invention is a real precipitation. By contrast, the reaction of alginate with Ba according to the prior techniques represents a cross-linking of the polymer chains. This cross-linking yields big molecules which are no longer soluble.

The inventive process distinguishes over the Zimmermann and Klöck references since the processes of these references start from dissolved commercial sodium or potassium alginate which is precipitated by barium or other multivalent cations to form an insoluble crosslinked complex. This complex is treated with acids at high temperature for purification. A disadvantage of this process is that the alginate is chemically and physically modified. The complex is then washed and treated with alcohol to dissolve alcohol-soluble contaminants. In a last step the water-insoluble alginate complex is dissolved with EDTA. By contrast, in the present invention, what is purified is an alginate extracted into solution, not a solid alginate. No acid or heat is used, and alcohol is used to precipitate the alginate from the solution. With the Zimmermann and Klöck process, already extracted alginate can be purified, but it is not possible to extract alginate from raw algae material. It should be noted that the present

invention is not simply a process for dissolving alginate with EDTA; the EDTA extraction is just one step in a series of specific purification measures.

Commercially available alginates, are often prepared from mixtures of different algae species leading to a huge charge variability, concerning the physical and chemical properties of the alginate (Orive et al. 2003; page 104, right column, second paragraph). Even if all the impurities are removable according to the prior inventions, the physical properties will vary from charge to charge. The present invention has the advantage that standardized material from single species can be used, leading to a reproducible product.

I declare that the foregoing is true and correct, that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 22.01.2004

Dr. Frank Thürmer